"Effect of Glass Walls on Thermionic Currents," by Saul Dushman.

"A New Design of Flicker Photometer for Laboratory Colored-light Photometry," by H. E. Ives and E. J. Brady.

"Note on the Physiological Effect of the Current," by F. J. Rogers.

"Examples of the Precision Attainable in Determinations of Thermal Expansivity," by Arthur W. Gray.

"Dioptric Formulæ for Combined Cylindrical Lenses at Oblique Axes," by Charles Sheard. (By title.)

"The Testing of Potentiometers," by Frank Wenner and Ernest Weibel.

"An Absorbing Solution for Eliminating Color Differences in Photometry," by H. E. Ives and E. F. Kingsbury.

"Photographs of Retrograde Rays, (*a*) from the Cold Cathode, (*b*) from the Hot Lime Cathode," by O. H. Smith.

A. D. Cole,
Secretary

THE SOCIETY OF AMERICAN BACTERIOLOGISTS

II

THURSDAY, JANUARY 1, 1914

Systematic Bacteriology

Morphology of the Bacteria (Vibro and Spirillum), An Early Research: Joseph Leidy, Jr.

This paper will be published in Science.

The Classification Card and the Type or Study which it Merits: H. A. Harding.

The classification card has not appealed to the pathologists, because they could test unknown cultures more quickly on animals, nor to water bacteriologists because their attention has been focused upon *B. coli* and special media, but it has been very valuable to students of bacterial ecology. The card is justly criticized because the observations of bacterial cultures are not always accurately recorded by it and because the present group number is unwieldy and of undetermined value as a basis for classification. One of the most urgent demands of bacteriology to-day is the careful testing out of various suggestions looking toward an improved technique. Dr. H. J. Conn has suggested that the ease of handling the group number can be increased by pointing it off into periods of about three figures each. The significance of such periods would be increased if each was devoted to a class of reactions such as morphology, fermentation, nitrogen relations and enzyms. The reactions should be selected for this group number after careful study of their accuracy and utility, and pending the results of this study the card of 1907 should be retained practically unchanged.

Constancy in the Fermentative Activity of Streptococci: Jean Broadhurst.

Attempts to correlate the fermentative results of different workers in saccharose mannit and other Gordon media have led to a series of experiments dealing with the different conditions characterizing the technique in different laboratories. Differences, such as acidity, presence or absence of sugars, subjection to raw and variously heated milks, were tried out without finding any definite results on the later Gordon reactions. Slight and brief increases in temperature (above 37°) depressed fermentative activity decidedly. Much more marked (as previously reported) were the contrasts resulting from the use of meat extract and of meat Gordon media. These effects are evidently not necessarily lasting. Permanent changes were effected by a stay in the alimentary canal (*e. g.*, a gain in lactose in dogs fed on streptococci-free milk). Cultures kept for 1 to 4 months on meat agar (10-day transfers) showed a gain in the amount of fermentative activity (the amount of acid), many strains show also a gain in the number of substances fermented; less often a loss occurred in the number of substances fermented. Studies carried on with individual animals for varying periods (2–10 mo.) showed unexpectedly wide ranges in the physiological types of mouth and fecal streptococci, affecting probably the diagnostic value of fermentative reactions. These, and other phases of the work reported upon, seem to warrant the following conclusions: (1) Constancy may be claimed for streptococci under identical or duplicate conditions. (2) Constancy in these fermentative responses is also characteristic of a large percentage of strains, under varying or varied conditions. Age, a stay in the alimentary canal, and meat extract, have more effect on such results than any of the (19) varied conditions tried.

The Relation of Habitat and Physiological Characters in the Streptococci: L. A. Rogers and Arnold Dahlberg.

It is reasonable to assume that true species in the bacteria will be found associated with a definite habitat as it is with the higher plants and animals. Studies were made of the physiological characters of 51 cultures from infected udders; 114 cultures from bovine feces; 31 cultures from

the mouths of cows and 42 cultures from milk obtained by selecting cultures showing chains in lactose bile at 37°. The morphology varied under different conditions but the udder cultures showed the most uniform and consistent chain formation. The cultures from the several sources differed in the amount of acid formed in dextrose broth, those from the mouth giving the highest acidity and those from the udder the lowest. The milk cultures were slightly higher than those from the udder. The udder cultures would be divided into two distinct groups, one of which fermented dextrose, saccharose and lactose and occasionally mannite and agreed with the published descriptions of strep. pyogenes. The second group frequently liquefied gelatin and in addition to dextrose, saccharose and lactose, usually fermented the alcohols mannite and glycerine. The cultures from feces were particularly uniform in their reactions, fermenting dextrose, saccharose, lactose and raffinose and frequently the polysaccharides starch and inulin, but failing to ferment the alcohols or liquefy gelatin. The cultures from the mouth differed from those from the udder in the higher percentages of raffinose, inulin and mannite fermenters and in less action on glycerine and gelatin. They are sharply differentiated from the feces organisms in their general failure to ferment starch and in the much higher percentage of mannite fermenters. Two of the milk cultures evidently belong with the feces group. All others may be placed in one of the two udder groups.

The Significant Characters of the Colon Group Isolated from Cow Feces: L. A. ROGERS, WM. M. CLARK AND ALICE C. EVANS.

Previous work on a collection of the colon type from milk demonstrated that the gas ratio and volume are constant under uniform conditions; that, on the basis of the gas ratio and volume, the cultures may be divided into two distinct groups and that the correlation of the fermentative ability with the gas ratio makes this distinction sharply defined.

This paper records the results of a similar study on 150 cultures isolated from bovine feces. None of these cultures liquefied gelatin and all but one found indol from tryptophane. By the use of a simple medium and exact methods of analysis it was found that in 149 cultures the CO_2:H_2 ratio varied from 0.98 to 1.20 only. One culture only gave a ratio identifying it with the high ratio group which made up 48 per cent. of the milk series. The 149 low ratio (0.98–1.20) cultures were readily divided into two groups, one of which fermented dextrose, saccharose, lactose, raffinose, mannite, glycerine and dulcite, but almost invariably failed to ferment starch, inulin and adonite, while the second group ferments dextrose, lactose, mannite and glycerine, occasionally ferments adonite and dulcite and fails to ferment saccharose, raffinose, starch and inulin.

These groups agree almost perfectly with two groups which may be formed from the low-ratio cultures isolated from milk. Special methods failed to give evidence, with the exception of the single culture mentioned, of the presence in bovine feces of the high ratio group which made up about one half of the milk collection.

Studies on the Classification of the Colon Group: I. J. KLIGLER.

Eighty organisms generally classed under the colon group were subjected to a series of fermentative and other tests with a view of determining their natural grouping as based on biometric principles. The following tests were employed: (1) Morphology, Gram. (2) Fermentation of dextrose, lactose, saccharose, raffinose, glycerine, mannite, dulcite, salicin and inulin. (3) Coagulation of milk. (4) Liquefaction of gelatin. (5) Production of indol. (6) Reduction of nitrates. (7) V. & P. reaction. Fifty-seven of the strains fell into the lactose fermenting division; twenty did not ferment lactose but fermented dextrose; three failed to ferment.

Acid production as determined by titrating aliquot portions of the broth with phenolphthalein as an indicator was found to be a more constant and a more reliable differential test than gas production, as ordinarily determined. The degree of initial acidity had no appreciable effect on the final acidity, which was quite constant, reaching its maximum on about the fourth day. The fifty-seven lactose fermenters attacked mannite, glycerine, saccharose, salicin raffinose, dulcite and inulin in the order named. Mannite, raffinose and inulin were found to be of minor or doubtful classificatory importance. Saccharose divides the lactose group into two distinct subgroups.

On subdividing the saccharose groups on the basis of dulcite and salicin fermentation respectively, it was found that the saccharose-salicin groups gave better correlations with indol production, V. & P. reaction and gelatin liquefaction, than did the saccharose-dulcite groups.

The saccharose positive, salicin positive group corresponds to ***B. aerogenes.***

The saccharose positive, salicin negative group corresponds to *B. communior.*

The saccharose negative salicin positive group corresponds to *B. communis.*

The saccharose negative salicin negative group corresponds to *B. acidi-lactici.*

Glycerine was found to be of value in separating the cloacæ forms from the aerogenes bacilli, 78 per cent. of the saccharose positive, salicin positive, glycerine negative strains being liquefiers.

It must be kept in mind, of course, that this classification was obtained with a relatively small number of organisms and can at best be considered only tentative. The results are, however, sufficiently interesting to merit further investigation, especially on the part of those interested in the bacteriology of water. Of the dextrose positive lactose negative forms five liquefied gelatine and fermented dextrose and saccharose but failed to ferment any of the other sugars, with the exception of glycerine, which was fermented by two of the organisms. Of the other tests, all were negative with the exception of indol, which was negative for the two glycerine positive organisms and positive for the glycerine negative. For the present all the five may be grouped under the name *B. vulgaris.* The sixty-two members of the colon group discussed may, therefore, be said to fall into six main species as follows:

Species	Specific Texts	No. of Organisms
B. communior	dex. +, lac. +, sac, +, sal. —,	12.
B. communis	..dex. +, lac. +, sac, —, sal. +,	11.
B. aerogenes	..dex. +, lac. +, sac, +, sal. +,	19.
B. acidi lactici	..dex. +, lac. +, sac, —, sal. —,	6.
B. cloacæ	....dex. +, lac. +, sac, +, sal. +, glyc. —,	9.
B. vulgaris	...dex. +, lac. —, sac, +, gel. +,	5.

A Biochemical Study of Proteins with Reference to the Behavior of Bacteria towards Pure Animal and Vegetable Proteins: JOEL A. SPERRY, 2D.

Solutions of unchanged egg albumin, serum albumin and edestin were carefully prepared. To these solutions sodium chloride, sodium sulphate, calcium chloride and potassium phosphate were added. The composition of these solutions was then of such a character that the bacteria were obliged to break down the protein molecule in order to obtain the necessary nitrogen to synthesize their food in the presence of the inorganic salts. The two most important factors in this investigation are: first, that the protein used is really a normal or unchanged protein, and second, that there must be nothing in the final solution from which the organism might obtain the necessary nitrogen, except the native protein. The organisms used in this investigation were *B. subtilis, B. prodigiosus, B. anthracis, B. proteus vulgaris* (two different strains), *B. proteus mirabilis, B. coli, B. typhi, B. pyocyaneus, Bacillus "Z,"* a proteus-like organism isolated from the feces of white rats fed on experimental protein diets, and the anaerobes, *B. putrificus, B. anthracis symptomatici,* and *B. edematis maligni.*

Test tubes containing about 10 c.c. of the protein solutions were inoculated from 24-hour slant agar cultures. In making the inoculations every precaution was observed to avoid transferring any of the medium to the tubes containing the protein solutions. Plates were poured immediately after inoculation, and at intervals of 24, 48, 96 hours, and one week; in a few instances ten days and two weeks. The amount of the inoculated material used in the plates was 0.5 c.c. of a 1:10,000 dilution. The plates were incubated at suitable temperatures and the number of colonies counted 24 to 48 hours later. The period of time during which the organisms survived after being planted in the protein solutions, as shown by the examination of the plates, varied from 48 hours to 10 days. None of the inoculated material gave any visible evidence of decomposition or even putrefaction. Flasks containing 25 c.c. of the different protein solutions were inoculated with various organisms and incubated at the optimum temperature for the organism under observation, for a period of two weeks. At the end of this time tests were made to determine the quantity of coagulable protein. The amount of coagulable protein in the inoculated flasks and the control flasks remained the same, showing clearly that there was no appreciable loss of protein.

A Study of the Bacteria Concerned in the Production of the Characteristic Flavor in Cheese of the Cheddar Type: ALICE C. EVANS AND E. G. HASTINGS.

A comparative study of the flora of raw- and pasteurized-milk cheese of the Cheddar type has been made, with reference, particularly, to the production of characteristic flavors. The raw-milk cheese flora was found to consist of the following four groups of cheese organisms: *Bact. lactis acidi, Bact. casei,* Streptococci and Micrococci. Several varieties of each group occur in

the cheese, according to the classification as determined by the fermentation in broth containing carbohydrates or related substances. The flora of pasteurized-milk cheese is shown to depend upon the organisms introduced in the starter, with the exception of the *Bact. casei* group, which develops slowly and is concerned with the production of the biting flavor in mature cheese.

Many experimental pasteurized-milk cheeses were made with starters consisting of the organisms isolated from normal raw-milk cheese, either in pure culture or in varying combinations. The results of these experiments showed that pronounced differences in the flavor could be brought about by varying the cultures in the starter. Certain combinations in the starter resulted in an improvement of the flavor.

Technic

The Application of Practical Records to the Maintenance of Stock Bacterial Cultures: L. T. CLARK AND W. L. DODD. (By invitation.)

The scope of a bacterial culture bureau has been gradually broadened, as new organisms have been isolated requiring additional tests to differentiate between old and new species. To assist in their classification a practical system of records is a necessity and to this end the writers submit a method which has had three years' application. Essentially it consists of a double system of records: one, a card index, consisting of classification charts which provide for the morphological, cultural and biochemical characteristics as well as space for the name, age, source and number of the organisms in question. The auxiliary system is a book record of the cultures in numerical rotation.

To avoid confusion resulting from maintaining cultures on the shelves in numerical sequence, an arrangement in groups according to biochemical, morphological and cultural characteristics has been found convenient. Protection from light and dust is provided by means of the black boxes described by Novy. The variable requirements of organisms make it necessary to transplant them at intervals ranging from three weeks to a week or less. Growth of stock anaerobes is established in a volume of pure hydrogen gas. Most pathogenic types may be grown for the first twenty-four hours at incubator temperature, after which they are easily maintained at ordinary room temperature. Saprophytic cultures are kept continuously at room temperature. A duplicate set of cultures is maintained at a low temperature.

The following advantages may be claimed for the system as outlined: The history of the culture forms a part of the record and is readily available; the cultures are easily handled and transplanted; any one of the organisms may be easily and quickly located by its number, name or by some predominant biologic characteristic.

The arrangement in groups, of cultures of similar characteristics, facilitates a further and more complete classification. This leads to the detection of variations between strains of the same species.

A Study of the "Tellurite Reaction" with the Colon-typhoid Group and other Organisms: LEWIS DAVIS.

The writer has investigated the reaction of potassium tellurite with the more important members of the colon-typhoid group and allied organisms, with the view of determining:

(*a*) Differences in antiseptic action on the various members of the group; (*b*) variations in the macroscopical appearance, character and velocity of the "tellurite reaction" under optimum conditions; (*c*) the influence of treatment with tellurite on the biochemical activities of the organism.

The bacteria studied included the following, arranged in the order of their resistance to the antiseptic action of potassium tellurite: *B. capsulatus mucosus, B. capsulatus, B. coli communis, Bact. (lactis) aerogenes, B. cloacæ, B. proteus vulgaris, B. paratyphosus "B," B. of swine plague, B. enteritidis, B. typhosus, B. paratyphosus "A," B. paracolon, B. acidi lactici, B. choleræ suis, B. rhinoscleromatis, B. pneumoniæ, Bact. dysenteriæ (Shiga), B. icteroides, Bact. dysenteriæ* (Flexner) and *B. zopfii.* The variations in resistance to potassium tellurite, as well as in the appearance of their reaction with this salt, are considered sufficient to suggest the use of the tellurite for differential diagnosis within the colon-typhoid group.

The intensity of bacterial action on potassium tellurite was found to depend upon the individual resistance of the bacterium and the concentration of the salt present. The velocity of reduction of the tellurite is considered to be a specific function of an organism apart from its resistance to antiseptic action. Most of the types studied showed a reaction within thirty minutes. With the colon bacillus the reaction was almost instantaneous. Comparison was made of the action of "tellurited" and normal bacteria with dextrose, lactose and saccharose bouillons, respectively, as well

as with litmus milk. Treatment with potassium tellurite was found to have practically no influence on the biochemical reactions of an organism.

An Improved Technique for Performing the Grüber-Widal Test for Typhoid Fever: F. M. Meader, M.D.

The method herewith described is not new in its elements, but the combination is one which I have not noticed in other laboratories that I have visited or in the literature. We have been using the method in the city laboratory of Syracuse for three months now with considerable satisfaction. A description of the method is herewith offered.

Apparatus.—There are used the following materials: 1st, a vial with straight sides, 2 cm. long and having a 0.5 cm. lumen. The cork stopper has fixed into the inner surface a lance. (*A*) 2d, a standard wire loop made from a No. 25 U. S. gauge platinum wire, the lumen of which is 2 mm. in diameter. (*B*) 3d, a capillary pipette with perforated nipple, graduated in two places to deliver the equivalent of 3 and 4 standard loopfuls of serum. (*C*) 4th, a mixture tray having a dozen or more cells such as is used by artists for mixing paints. (*D*) 5th, a hanging drop slide. (*E*) 6th, cover slips. (*F*) 7th, alboline oil. 8th, sterile salt solution, 9th, a 24-hour bouillon culture of *B. typhosus.*

Procedure.—Prepare a control by placing a loopful of salt solution upon a coverslip. Then mix with it a loopful of the culture of *B. typhosus.* Place a ring of alboline oil around the depression in the cover glass. Invert the coverslip over the depression in the slide so that the center of the drop comes over the middle of the depression. Examine under the microscope to see that the culture is active and that the organisms are sufficiently numerous. If the preparation is satisfactory, the time is noted and it is set aside for one hour.

Given a sample of blood in vial *A.* If the vial is about one fourth full of blood, and is then centrifugalized, there will be a satisfactory amount of serum present. With the graduated pipette measure 4 loopfuls of salt solution into one of the cells of the mixing tray. Then measure 3 loopfuls of salt solution into another cell of the mixing tray. With the standard loop take one loopful of the serum and mix it with the salt solution first measured out. This makes the dilution of serum 1–5. Transfer a loopful of this mixture to the second solution measured out. This makes a dilution of 1–20. Transfer a loopful of this mixture to a coverslip. Add to it a loopful of the culture of *Bacillus typhosus.* This makes a dilution of 1–40. Prepare a hanging-drop slide as above with oil. Invert the coverslip over the depression and examine under microscope. Note the time and examine one hour later for agglutination.

The particular point of value in this technique is the use of the capillary pipette. Since there is a perforation in the nipple, the salt solution will rise to the graduations by capillary force, so that an exact amount of diluent can be easily and accurately obtained. The liquid can be pushed out into the cell of the diluting tray by covering the perforation with the finger and compressing the bulb. Since the viscosity of serum is greater than that of salt solution, the volume of a loopful of serum will be greater than the loopful of salt solution. So that in calibrating the pipette 3 and 4 loopfuls of serum should be used instead of salt solution in order that in practise the volumes of diluent and serum will be comparable. The method commends itself for its simplicity, rapidity of operation and precision in measurement.

A Synthetic Medium for the Determination of Colon Bacilli in Ice Cream: S. Henry Ayers and William T. Johnson, Jr.

In a study of the bacteria in ice cream we have endeavored to prepare a synthetic medium for the detection of colon bacilli. During the experiments 53 different combinations were tried. The most satisfactory medium was made as follows: Agar, 1.5 per cent.; asparagin, 0.3 per cent.; sodium dibasic phosphate, 0.1 per cent.; lactose, 1.0 per cent., and 2 per cent. of a saturated solution of litmus. The majority of the bacteria in ice cream did not grow on this medium, while colon bacilli showed quite characteristic acid colonies which with a little practise could be readily detected. The colon count on litmus lactose asparagin agar was compared with the estimated number from lactose bile tubes in 43 samples of ice cream. In 41 of the 43 samples the number determined on the plates was higher than the estimated number from the bile tubes.

Suspected colon colonies on the asparagin plates from 19 samples were picked off and inoculated into lactose broth fermentation tubes. From ten plates all the suspected colonies, or 100 per cent., proved to be gas formers. Among the other nine plates the percentages ranged from 87.17 per cent. to 98.01 per cent. This shows that it is possible to detect quite accurately any colonies of gas-forming bacteria on litmus lactose asparagin agar.

A comparison of this medium with Endo medium showed that the colon count on asparagin agar was much lower than that on the other medium. We found, however, that in some cases at least it was impossible to consider all typical colonies on Endo plates as colon bacilli. Certain strains of *B. coli* failed to give typical colonies on Endo plates and acid and peptonizing bacteria gave reactions similar to some of the colon strains.

It is evident that we have no entirely satisfactory method for the determination of colon bacilli, but it is believed that the use of synthetic media may be developed to a point where it will be superior to other methods.

A Satisfactory Platinum Needle: L. A. ROGERS.

The tendency of platinum needles set in glass handles to break when they are flamed, is a source of annoyance. A needle which will avoid this trouble may be made by fusing the platinum wire into a copper wire. This may be done by twisting a bit of small wire about the platinum wire and holding in the flame of a blast lamp until it forms a ball at the end of the wire. The copper ball and the end of a copper wire of the proper size are held together in the flame until they fuse. The rough joint obtained may be hammered or filed to approximately the diameter of the copper wire, which should be large enough to insure rigidity. The wire may be mounted in a capillary tube or in an ordinary glass tube with plaster of Paris. The needle may be thoroughly flamed without danger of breaking.

FRIDAY, JANUARY 2

Immunity

On the Value of a New Skin Test for Diagnosis of Tuberculosis: DR. J. BRONFENBRENNER.

In the work reported to this society last year by Dr. Manwaring and myself[1] and subsequently continued and published in the *Journal of Experimental Medicine*,[2] it was shown that tuberculous guinea-pigs acquire the power of reducing the number of tubercle bacilli injected into their peritoneal cavity; that certain fixed cells of the peritoneal cavity were apparently responsible for this phenomenon, as even removed from the body of the guinea-pig the isolated peritoneal tissues of tuberculosis animals had the power of reducing the number of tubercle bacilli placed in contact with them; that, however, as far as our experiments went the intraperitoneal destruction of tubercle bacilli in tuberculous animals was not caused by circulating antibody. It was thought, however, worth while to investigate what changes the blood of these guinea-pigs underwent in the conditions of the experiment, as it seemed improbable that the cells of the peritoneal cavity could have acquired immune properties without these being present in the blood. A series of experiments was undertaken to test complement deviation on the blood of tuberculous animals, but the results obtained varied with the different strains of tubercle bacillus used as antigen. In general, however, experiments showed that the blood of guinea-pigs often contains specific antibody against tuberculous antigen. Having established this fact, an attempt was made to see if this antibody is of the nature of a bacteriolysin. The series of experiments were performed both in vitro and in vivo. In the course of this last series of experiments a very interesting phenomenon was noticed, namely: that if a normal guinea-pig was injected intraperitoneally with a mixture of the serum of a tuberculous guinea-pig with the peritoneal exudate resulting from the injection of a small amount (10,000) of tubercle bacilli in the peritoneal cavity of another highly immunized guinea-pig, often a local reaction would result on the spot of inoculation, followed by a rise of temperature. This local reaction was especially pronounced in the cases where the peritoneal wall was punctured several times for the purpose of removing a sample of the exudate, and in this way probably a part of the mixture was introduced from within the peritoneal cavity under the skin of the animal. In analyzing this phenomenon it was found that the peritoneal exudate employed in these experiments could be conveniently replaced by a crude tuberculin as prepared by the Board of Health of New York, but not very well by a suspension of washed tubercle bacilli. The non-washed (possibly partly autolyzed) suspension of tubercle bacilli, especially if not freshly prepared, could also be used successfully. Since then, a number of tests were performed in which guinea-pig serum was replaced by the serum of tuberculous patients, and it was found that the reaction, although not very constant, is of a prognostic value in tuberculosis.

While the work is still in progress, the experiments performed up to date seem to show that the complement is an important factor in the phenomenon, inasmuch as heated sera failed to give this reaction, yet if activated by the addition of a complement and left at room temperature for a

[1] *Centrbl. f. Bact. Ref.*, Bd. 59, No. 12, p. 371.

[2] *Jour. of Exp. Med.*, 1913, Vol. XVIII., No. 6, p. 601.

short time, they could be reactivated. Whether the reaction is due to the liberation of an anaphilatoxin from the mixture of the serum containing the toxogenin and complement with the antigen of the tuberculin, is a question to be decided in the experiments which are to follow. At present I wish to call attention to this phenomenon as a possible method for the diagnosis of tuberculosis at least in cases where the condition is not too far advanced and where there is some of the free antibody in the circulating blood.

Subcutaneous injection 0.55 c.c. of a mixture of fresh blood of patients suffering from tuberculosis (1 c.c.) with tuberculin (crude diluted 1 to 10 0.1 c.c.) into a normal guinea-pig causes a local reaction similar in its aspect to a tuberculin reaction which is of good prognostic value in diagnosis of tuberculosis.

The Relationship of Anaphylaxis to Immunity: Fraser B. Gurd.

The experiments reported in this paper were undertaken in the hope of procuring data which might throw light upon the fact, now well established, that in order that an animal may become "immune" to a complex proteid, such as serum albumin, it is necessary that the animal first become, potentially at least, anaphylactic.

The author believes that there is not sufficient evidence upon which to base the theory of sessile and free receptors, as suggested by Friedberger and recently actively championed by Weil. That the reaction of the body to the parenteral introduction of foreign proteins is an exhibition of the property of parenteral digestion, is well established, as indicated by the work of Alberhalden, Zunz, Friedberger and others. That at one stage in the process of protein cleavage a highly poisonous split product is produced, is also proven, and that it is on account of the elaboration of ferments or lysins, capable of producing cleavage of the injected protein with the liberation of this toxic product, that the anaphylactic state is developed, appears sufficiently well supported by numerous experiments. The author desires to suggest that the immunity to or tolerance of heterologous protein introduction is due to the tissues of the repeatedly injected animal acquiring the property of elaborating a second order of lysins which are potent to produce more complete cleavage of the toxic split-protein products. Thus it is due to the presence in the body fluids and tissues of two orders of lysins, that the "immune" animal is itself protected against the harmful effects of protein injection, even though its serum is potent to passively sensitize normal animals.

The author's experiments prove that it is possible, by varying the quantity of transferred serum injected, to render normal animals either highly sensitive or "immune." Thus, two guinea-pigs which received (intravenously) .6 c.c. of serum from an immune rabbit were found to be sensitive (at the end of 24 hours), to 5 and 6 minims of sheep's serum, the latter with a fatal termination; whereas a rabbit which received 4.0 and 4.5 c.c. of the same rabbit serum on two successive days, and 24 hours later was injected with 7 minims of sheep's serum, was found to be immune.

In another series of experiments the toxic injections were carried out immediately following the injection (intravenous) of the transferred serum. In these experiments it was found that whereas 0.5 c.c. of rabbit's serum rendered a normal pig highly anaphylactic (dyspnea and convulsions), the injection of 2.75 c.c. was sufficient to induce a complete tolerance to the toxic injection of the protein.

Study of the Bacteriology of the Posterior Nasopharynx in Scarlatina: N. S. Ferry, M.D.

The study of the bacteriology of the posterior nasopharynx in scarlatina was undertaken by the writer to isolate, if possible and determine the rôle of a certain micrococcus found in this region and briefly described by Dr. Schultze in a preliminary report in the *Medical Record,* New York, December 10, 1910. This organism was seen by Dr. Schultze in smears from 459 out of 555 cultures taken from the throats of patients suffering with typical symptoms of the disease. The greatest number of positive findings have been obtained by swabbing the posterior pharyngeal wall and allowing the swab to stand in a test tube of bouillon a few hours. The entire amount of bouillon is then plated in the usual manner. The organism was not isolated in the later stages of the disease and was not found in any of the purulent discharges nor the blood, which seems very significant considering the fact that it appears to coincide with the contention of the majority of observers that the disease is contagious only in its early stages. For convenience in nomenclature this organism was called by the writer Micrococcus "S" and, for the present, it will continue to be designated by that term.

The Mic. "S" is a large coccus usually found in pairs and often tetrads which grows luxuriantly on all culture media after the first few generations. Whether the Mic. "S" has or has not any spe-

cific clinical or pathological significance, for the purpose of placing it on record, a detailed description of the organism will be given, followed by a brief review of the experimental work carried out with it.

Morphology.—Mic. "S" is a large, clearly defined, biscuit-shaped diplococcus, sometimes appearing in tetrads, measuring about the size of the meningococcus and gonococcus. It is non-spore-bearing, non-motile, non-capsulated, stains deeply with all aniline dyes and is positive to Gram.

Cultural Reactions

Agar Slant.—Abundant, smooth, grayish-white, glistening, opaque, filiform or slightly beaded raised growth, becoming somewhat viscid within a few days.

Agar Deep.—Abundant, filiform, growth usually in one plane, with a slightly spreading surface growth.

Agar Colony.—Slow-growing, round, smooth, convex, entire, coarsely granular colony.

Bouillon.—Slight growth, clear with sediment.

Potato.—Very slight, colorless growth.

Litmus Milk.—No change.

Koch's Serum.—Slight, filiform, white growth.

Loeffler's Serum.—Abundant, filiform, smooth, glistening, pinkish white growth.

Gelatin Stab.—Gradual stratiform liquefaction. In about five days a cup forms at the surface and as liquefaction increases it reaches the sides of the tube and then proceeds downwards. At the end of six weeks, the medium is liquefied about half way down.

Indol negative.

Litmus Sugars.—Glucose, maltose and saccharose gave an acid reaction; galactose, levulose and lactose, no change.

Pathogenic Powers

While extensive inoculation experiments were carried out with this organism, nothing of any special specific significance could be gleaned from the work. Whether the organism soon loses its virulence on artificial culture media, or whether it is devoid of all pathogenic properties for the animals used, is a question which was undecided. Believing, nevertheless, that this organism was found in a large enough proportion of cases to warrant further work, irrespective of the apparently negative pathogenicity, several vaccines were prepared with it which have been fairly tested out, both prophylactically and therapeutically under the supervision of Dr. Schultze in New York and Dr. Kiefer in Detroit, which work will be reported in later communications by them.

Other observers have, from time to time, noted the presence of a large diplococcus in cultures and smears from the throats of scarlatina cases, and yet no one has succeeded in proving that it has any part to play in the disease and, therefore, very little stress has been laid on the findings. The organism described by these writers is so varied in its morphology and cultural characteristics that one is inclined to one of two suppositions. Either it is extremely polymorphous, as was claimed by Class, or else they were dealing with cultures containing streptococci, which organisms may often be seen, in smears from the throat directly or from cultures, as large diplococci.

From the experience of the writer with the organism he has isolated and named Mic. "S," which tallies with the general descriptions given by Schultze, Class and others, as seen in smears it is quite essential not only to plate the cultures, but to be absolutely certain that the colonies from which the cultures are taken have no small colonies of streptococci, either deep or superficial, adjacent to or near them. This can only be ascertained by examining the field with a lens. Among the several known organisms found in the throat, from which the Mic. "S" should be differentiated, the following are the most important.

Mic. catarrhalis.
Mic. tetragenus.
Mic. pharyngis siccus.
Dip. intracellularis meningitidis.

Studies in Avian Tuberculosis: L. R. HIMMELBERGER.

Cultures of the avian tubercle bacilli were grown on sterile banana and glycerinated slants of carrots, turnips and garden beets. The different character of the growths obtained on the different media, would suggest the use of these vegetables as differentiating media. Attempts were made to infect white rats, under conditions simulating cohabitation with tuberculous chickens and guinea-pigs and rabbits by injection of pure cultures subcutaneously and intravenously (in rabbits) without success. Two calves were infected by the ingestion of mascerated tubercular organs of chickens while in one an attempt at infection by ingestion was unsuccessful. The calves were tuberculin tested previous to infection and came from a cow which had given no actions to the tuberculin test, and were fed from a herd free from tuberculosis as indicated by repeated tuberculin tests. The calves which were infected gave reactions to tuberculin prepared from the avian organisms and on autopsy lesions of tuberculosis were found. It is regrettable that we were not able to isolate this organism. The agglutination test was tried on a limited number of birds both normal and diseased. In only

one case did a normal bird (as shown by macroscopical examination at autopsy) exhibit an agglutination titre over 1:50 and this bird had been subjected to infection. All of the birds showing lesions on post-mortem examination gave a titre as high as 1:100.

JANUARY 2, 1914

Pathology

A Comparative Study of the Intestinal Flora of White Rats Kept on Experimental and on Ordinary Mixed Diets: LEO F. RETTGER AND GEORGE D. HORTON.

The investigation extended over a period of almost one year, and was carried on in connection with the pure protein nutrition experiments of Osborne and Mendel. The feces of 22 rats were examined, 17 of the rats receiving the experimental diets consisting essentially of purified animal or vegetable protein, protein-free milk, starch and lard. The remaining 5 rats received ordinary mixed food consisting of sunflower seeds, carrots, dog-bread, meat, etc.

A change in the intestinal flora became apparent very soon after the rats were transferred from the ordinary to the special diets. The flora became more simplified, very few types being found after the first three or four days, as a rule. An increase in the number of Gram-positive organisms from 35–50 per cent. to 85–100 per cent. was frequently observed. There was no appreciable difference in the results, in so far as the individual proteins were concerned, with the exception of Zein. Although they were present in the feces of the stock-room rats in relatively large numbers, two organisms which are a part of, or closely related to, the acidophilus group of bacteria, were frequently present to the exclusion of all other types, except *Bacillus bifidus* of Tissier and *B. coli*, *B. bifidus* was much more abundant in the experimental rats than in those receiving the usual diet, while the number of *B. coli* was greatly reduced. No definite relationship could be established between the bodily conditions (growth, vigor, etc.) of the rats receiving the special diets, and the intestinal flora.

Anaerobic Culture of Coccidiodes Immitis: WARD J. MACNEAL AND RICHARD M. TAYLOR, M.D.

Two strains of *Coccidioides immitis* of Rixford and Gilchrist[3] have been studied, one derived from a fatal case of generalized infection which occurred in the practise of Dr. Chas. A. Powers, of Denver, and which has been studied by Whitman,[4] and a second isolated at this laboratory[5] from a similar case which occurred in the practise of Dr. Robt. T. Morris, of New York. We observed the metamorphosis of the spherical (Coccidioidal) bodies into typical mycelial growth on agar and the inverse change of the threads back into spherical bodies in the animal body. Finally by inoculating the spherical bodies into tubes of ascitic fluid containing bits of sterile animal tissue, or better, tubes of gelatinized horse serum, and covering these with paraffin oil or incubating in an atmosphere of hydrogen, we succeeded in obtaining abundant multiplication of the spherical form *in vitro*. The forms of the organism in these cultures resemble very closely those seen in diseased tissues.

Further Observations of the Thompson-McFadden Pellagra Commission upon the Etiology of Pellagra: J. F. SILER, P. E. GARRISON AND W. J. MACNEAL.

Information concerning the age and sex, occupations, location of domicile, general dietary habits, and concerning the existence of pellagra was obtained upon about five thousand persons by a house-to-house canvass of six cotton-mill villages. A similar study was carried out in one rural district of four square miles in which several cases of pellagra had occurred. Many other communities were studied in less detail. There was no definite relation observed between the occurrence of pellagra and the use of any particular foods. New cases developed for the most part in the immediate vicinity of old cases or after close association with them. In districts completely equipped with water-carriage systems of sewage disposal, we found pellagrins who had acquired the disease before moving to these districts. Cases apparently originating in these sewered districts were extremely rare and their origin there somewhat doubtful. These observations strongly suggest that unsanitary methods of sewage disposal have an important relationship to the spread of pellagra. If these indications can be confirmed in other places, we feel that the proper correction of these conditions by the installation of water-carriage systems of sew-

[3] Rixford, Emmet, and Gilchrist, T. C., "Two Cases of Protozoon (Coccidioidal) Infection of the Skin and Other Organs," Johns Hopkins Hospital Reports, 1896, Vol. I., p. 209–290.

[4] Whitman, R. C., "A Contribution to the Botany of the Organism of Blastomycosis," *Jour. of Infectious Diseases*, July, 1913, Vol. XIII., pp. 85–95.

[5] MacNeal, W. J., and Hjelm, C. E., "Note on a Mold, *Coccidioides immitis*, Found in a Case of Generalized Infection in Man," *Jour. Amer. Med. Assoc.*, December 6, 1913, Vol. LXI., No. 23, p. 2044.

age disposal will go far toward restricting the spread of the disease. The exact mode of transmission of pellagra is still uncertain and we strongly urge the continued study of food contamination, of insects as transmitting agents and of close personal association as possible factors in its spread.

Further Studies with Reference to Spirochæta suis: WALTER E. KING, RAYMOND H. DRAKE AND G. L. HOFFMAN.

This report gives in detail the results of the study of the flora of the crypts in the ceca, intestinal ulcers and the external local lesions of a number of normal, immune and diseased hogs, with reference to the presence of *Spirochæta suis*. The study includes the examination of 234 cases by means of the dark field. Of these, positive findings have been made in 171 cases, negative findings in 63 cases. Of the latter, 38 cases were hogs immune to cholera, 2 were animals susceptible but not exposed to hog cholera, 3 with typical hog cholera but treated with toxic doses of mercuric or arsenical preparations, 16 in which the organism was found either in local lesions or in the crypts and ulcers of the intestines, and 6 cases resulting in negative control findings. In 5 hogs which were made immune to cholera, *Spirochæta suis* was found in the crypts of their ceca at intervals of from 10 days to 11 weeks after exposure.

These data, together with results already reported, warrant the tentative deduction of the following conclusions: (1) In the ulcerated areas of cecal mucosa and in the crypts, near the ileo-cecal valve, of hogs dead from cholera, is localized a constant species of spirochete, *Spirochæta suis*. Experimental evidence shows that this organism is pathogenic for swine and that it plays an important part in the production of hog cholera. (2) The crypts in the ceca of activity immunized hogs may sometimes contain *Spirochæta suis* for a variable period of time after immunization. (3) *Spirochæta suis* becomes localized in the necrotic tissue or purulent exudate of the external lesions, which are sometimes present in cases of typical hog cholera, especially of the subacute chronic types.

The Relation of Lavatory Appliances to the Spread of Intestinal Infections: B. R. RICKARDS AND L. B. CLORE.

These experiments were carried out to determine the rôle played by the chain pull and other appliances of the toilet room in transmitting from hand to hand typhoid bacilli and other organisms capable of causing intestinal disorders. The surfaces tested were rubbed with sterile cotton swabs previously moistened with sterile water. Plates made by rubbing the swabs over the surface of Endos medium in Petri dishes. The plates were incubated for 48 hours at 37° C. and inoculations into plain broth were then made from a number of each of the various kinds of colonies found, attention being centered on those cultures having a typical *Bacillus coli* appearance. The work was confined entirely to the detection of the colon group, lack of time preventing experiments being carried on for the presence of *B. ærogenes capsulatus*. The broth cultures were examined microscopically after 48 hours' incubation and transplants made into Hiss's semi-solid media. These tests were carried out on media containing, respectively, dextrose, lactose, saccharose and mannit. Typical growth on all four media was taken to mean that the organism isolated was a member of the typhoid-colon group.

In each instance swabs were taken from the front of the seat, back of the seat, door knob and from the handle of the device operating the flushing tank. From the low flush tank type, cultures were made from the metal or porcelain lever. If, of the high box type, the swab inoculation was made from the metal or porcelain handle and oftentimes from the lower parts of the chain, the object of the latter being to see if there was any attempt by the users of the closets to avoid infection by putting the hands on that part of the apparatus not commonly used. For the same reason swabs were in all cases taken from portions of the door with which the hand might come in contact in case the handle of the door was avoided.

B. coli was isolated in pure culture from swabs taken from the following locations. (1) From the seat in two different toilets of the scientific department of the manufacturing establishment. In one case tests were made at three different times and *B. coli* found each time. (2) From four different seats in the public comfort station. In no case was *B. coli* detected on the handles of pull or push levers nor on the chains, nor was this organism detected on the metal or porcelain door handles or upon the wood of the door.

While the results by experiments fail to show presence of *B. coli* on any other surfaces except on the wooden seat, we still feel that there is a possibility that the handles of flushing devices may at times serve as a means of carrying typhoid or other intestinal infection or possibly gonorrhea or syphilis from hand to hand, at short intervals and that foot or automatic levers should take their places.

The Malarial Parasites: MARY R. LAWSON, M.D.

Many of the misconceptions in regard to the morphology and biology of the malarial parasites are due to the fact that the majority of observers have believed them to be intracellular, and that each parasite grew up and completed its life cycle within a single corpuscle, the segmentation of the parasite corresponding to the final destruction of the corpuscle. The writer believes the parasites to be extracellular throughout their existence, that is, when not in migration, they attach themselves to the external surface of the red corpuscles by means of protoplasmic pseudopodia surrounding mounds of corpuscular substance, which the parasite has "squeezed up" for the purpose of attachment. This interpretation is confirmed by seeing the corpuscular mounds at the periphery of the red corpuscles encircled by the pseudopodia of the parasites.

The evidence in favor of migration is:

1. The destruction of red corpuscles is usually out of all proportion to the number of parasites present, providing one parasite destroys but one corpuscle.
2. In multiple infection of red corpuscles by several young parasites, they can not all grow up on one corpuscle, therefore they must migrate or die.
3. Stages of parasitic migration such as (*a*) Free pigmented parasites, compact, amœboid, with pseudopodia. (*b*) Pigmented parasites attached to apparently healthy corpuscles. (*c*) Pigmented parasites (24 hr.) apparently in the act of abandoning degenerated corpuscles. (*d*) Parasites on corpuscular skeletons. (*e*) Corpuscular skeletons which are expanded remnants of red corpuscles which have been dehemoglobinized.

The sexual cycle takes place in the blood of man in the various malarial infections. The flagella are always derived from the chromatin substance, and from the chromatin alone. In the æstivo-autumnal infections the writer has observed but one flagellum to each crescent, while in the tertian and quartan infections, there are several flagella to each parasite.

A Preliminary Communication on the Etiology of Pyæmic Arthritis in Foals: FRANK W. SCHOFIELD.

The author after a brief discussion relative to modes of infections points out that intrauterine infection of foal can alone account for some cases, and most probably does for more than is generally believed. The bacteriology of the disease is reviewed and author's findings given. An organism of the colon typhoid group has been recovered uncontaminated from blood and joints in early stages of disease. The relationship of this organism to the disease was established by complement fixation tests using foals' blood and organism isolated as antigen. Positive fixation tests were also obtained from the blood of dams that have delivered foals which subsequently became diseased.

Experiments Bearing on Pulmonary Infection: FRANK W. SCHOFIELD.

Mention is made of two existing views regarding pulmonary infection. That it is due to direct inhalation of course into smaller air passages, or arises as the result of a primary infection of blood stream. The difficulty of infecting the lung by direct inhalation was demonstrated by experiments of following nature.

First Experiment.—Horses were exposed to a very fine spray from powder atomizer, the material used being equal parts gentian violet and powdered charcoal. After a few minutes' spraying the atmosphere became saturated with this fine violet powder. Horses breathing normally filtered all the powder out of the inspired air before same reached trachea. When excessive, labored and rapid breathing was induced the powder could be detected in the larger bronchi.

Second Series of Experiments.—A spray of *B. prodigiosus* was here substituted for the powder. The spray was manipulated so that the fine terminal portion of the same enveloped the nostrils of animals breathing it. The spray was kept up for several minutes. In most cases the organism could not be recovered past the larynx. When present in the trachea bacilli were few in numbers. The last experiment consisted of taking a number of swabs from the trachea of normal horses, cattle, sheep. In most cases the tracheæ were found to be sterile. When the organ was infected the organisms were *S. aureus, S. albus* and *Streptococci.* None of the common organisms present in the air these animals were breathing were recovered from the trachea.

Conclusions.—In health the trachea if not sterile has no constant bacterial flora. This could not be so if dust with bacteria could easily pass the nasopharynx.

That with nasal breathing most of the bacteria inhaled are removed before the air enters the trachea, even when the atmosphere is saturated with bacteria.

That direct infection of lung through nasal inspiration is almost impossible, under ordinary conditions.

A. PARKER HITCHENS,
Secretary